```
FILE 'REGISTRY' ENTERED AT 08:44:35 ON 19 DEC 2003

=> S NUCLEASE/CN
L1            1 NUCLEASE/CN

=> D

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS on STN
RN   9026-81-7  REGISTRY
CN     ***Nuclease (9CI)***     (CA INDEX NAME)
OTHER NAMES:
CN   Nucleic acid hydrolase
MF   Unspecified
CI   MAN
LC   STN Files:   ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA,
       CAPLUS, CEN, CHEMCATS, CIN, CSCHEM, EMBASE, IFICDB, IFIPAT, IFIUDB,
       NAPRALERT, PROMT, TOXCENTER, USPAT2, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
            2304 REFERENCES IN FILE CA (1907 TO DATE)
              51 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
            2310 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> S ENDONUCLEASE/CN
L2            1 ENDONUCLEASE/CN

=> D

L2   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS on STN
RN   9055-11-2  REGISTRY
CN   Nuclease, endo- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Cobra venom endonuclease
CN     ***Endonuclease***
MF   Unspecified
CI   MAN
LC   STN Files:   ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA,
       CAPLUS, CEN, CIN, CSCHEM, EMBASE, PROMT, TOXCENTER, USPAT2, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
            1425 REFERENCES IN FILE CA (1907 TO DATE)
              18 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
            1427 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> S RESTRICTION ENDONUCLEASE/CN
L3            1 RESTRICTION ENDONUCLEASE/CN

=> D

L3   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS on STN
RN   9075-08-5  REGISTRY
CN   Nuclease, restriction endodeoxyribo- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   136: PN: WO03072819 TABLE: 2 claimed sequence
CN   DNA restriction endonuclease
CN   DNA restriction enzyme
CN   E.C. 3.1.21.4
CN   E.C. 3.1.4.32
CN   Nuclease, deoxyribonucleic restriction endo-
CN   Restriction endodeoxyribonuclease
CN     ***Restriction endonuclease***
CN   Restriction enzyme
DR   37288-31-6
MF   Unspecified
CI   MAN
LC   STN Files:   ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS, CA, CAPLUS, CEN,
       CHEMLIST, CIN, CSCHEM, IFICDB, IFIPAT, IFIUDB, PROMT, TOXCENTER, USPAT2,
       USPATFULL
     Other Sources:   TSCA**
```

```
     (**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
          3545 REFERENCES IN FILE CA (1907 TO DATE)
            14 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
          3549 REFERENCES IN FILE CAPLUS (1907 TO DATE)


FILE 'CAPLUS' ENTERED AT 08:45:24 ON 19 DEC 2003

=> S L1 OR NUCLEASE;S L2 OR ENDONUCLEASE;S L3 OR RESTRICTION(W)(ENZYME OR ENDONUCLEASE)
          2311 L1
         19238 NUCLEASE
          6022 NUCLEASES
         23265 NUCLEASE
                 (NUCLEASE OR NUCLEASES)
L4       23343 L1 OR NUCLEASE


          1427 L2
         24433 ENDONUCLEASE
          7533 ENDONUCLEASES
         28482 ENDONUCLEASE
                 (ENDONUCLEASE OR ENDONUCLEASES)
L5       28521 L2 OR ENDONUCLEASE


          3549 L3
         87999 RESTRICTION
         11679 RESTRICTIONS
         98921 RESTRICTION
                 (RESTRICTION OR RESTRICTIONS)
        692261 ENZYME
        399085 ENZYMES
        871467 ENZYME
                 (ENZYME OR ENZYMES)
         24433 ENDONUCLEASE
          7533 ENDONUCLEASES
         28482 ENDONUCLEASE
                 (ENDONUCLEASE OR ENDONUCLEASES)
         29123 RESTRICTION(W)(ENZYME OR ENDONUCLEASE)
L6       29401 L3 OR RESTRICTION(W)(ENZYME OR ENDONUCLEASE)

=> S NICK;S NICKASE
          5354 NICK
          1025 NICKS
L7        6158 NICK
                 (NICK OR NICKS)


            45 NICKASE
             8 NICKASES
L8          49 NICKASE
                 (NICKASE OR NICKASES)

=> S SUBUNIT
        130969 SUBUNIT
         87967 SUBUNITS
L9      174383 SUBUNIT
                 (SUBUNIT OR SUBUNITS)

=> S L6 AND L8
L10         12 L6 AND L8

=> D 1-12 CBIB ABS

L10  ANSWER 1 OF 12  CAPLUS  COPYRIGHT 2003 ACS on STN
2003:734657   Document No. 139:241306  Methods and kits for detection of
     nucleic acids using fluorescence resonance energy transfer.  Rabbani,
     Elazar; Stavrianopoulos, Jannis G.; Donegan, James J.; Coleman, Jack; Liu,
     Dakai (Enzo Life Sciences, Inc., USA).  Eur. Pat. Appl. EP 1344835 A2
```

20030917, 115 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK. (English). CODEN: EPXXDW. APPLICATION: EP 2003-4895 20030306. PRIORITY: US 2002-96076 20020312.

AB This invention provides for compns. for use in real time nucleic acid detection processes. Such real time nucleic acid detection processes are carried out with energy transfer elements attached to nucleic acid primers, nucleotides, nucleic acid probes or nucleic acid binding agents. Real time nucleic acid detection allows for the qual. or quant. detection or detn. of single-stranded or double-stranded nucleic acids of interest in a sample. Other processes are provided by this invention including processes for removing a portion of a homopolymeric sequence, e.g., poly A sequence or tail, from an analyte or library of analytes. Compns. useful in carrying out such removal processes are also described and provided.

L10 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN

2003:571113 Document No. 139:129070 Assembly and purification of double-stranded polynucleotides from oligonucleotides comprising ***restriction*** ***endonuclease*** restriction sites. Frey, Gerhard; Short, Jay M.; Parra-Gessert, Lilian (Diversa Corporation, USA). PCT Int. Appl. WO 2003060084 A2 20030724, 159 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US1189 20030114. PRIORITY: US 2002-PV348609 20020114; US 2002-PV348761 20020114; US 2002-PV348764 20020114; US 2002-77474 20020214.

AB The invention provides methods for identifying and purifying double-stranded polynucleotides lacking base pair mismatches, insertion/deletion loops, and/or nucleotide gaps. The invention provides libraries of nucleic acid building blocks and methods for generating any nucleic acid sequence, including synthetic genes, antisense constructs, and polypeptide coding sequences. The libraries comprise pre-made multicodon (e.g., dicodon) oligonucleotide building blocks comprising ***restriction*** ***endonuclease*** restriction sites (e.g., Type IIS ***restriction*** ***endonuclease*** restriction sites), wherein the ***restriction*** ***endonuclease*** cuts at a fixed position outside of the recognition sequence to generate a single-stranded overhang. Purifying double-stranded polynucleotides lacking nucleotide gaps, base pair mismatches, and insertion/deletion loops comprises specific binding by selected DNA repair enzymes, such as Taq MutS and/or DNA glycosylases. The invention also provides chimeric antigen-binding mols. (e.g., single-chain antibodies) and the nucleic acids that encode them.

L10 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN

2003:539744 Document No. 139:272652 Pseudocomplementary PNAs as selective modifiers of protein activity on duplex DNA: the case of type IIs ***restriction*** ***enzymes*** . Protozanova, Ekaterina; Demidov, Vadim V.; Nielsen, Peter E.; Frank-Kamenetskii, Maxim D. (Center for Advanced Biotechnology, Boston University, Boston, MA, 02215, USA). Nucleic Acids Research, 31(14), 3929-3935 (English) 2003. CODEN: NARHAD. ISSN: 0305-1048. Publisher: Oxford University Press.

AB This study evaluates the potential of pseudocomplementary peptide nucleic acids (pcPNAs) for sequence-specific modification of enzyme activity towards double-stranded DNA (dsDNA). To this end, the authors analyze the ability of pcPNA-dsDNA complexes to site-selectively interfere with the action of four type IIs ***restriction*** ***enzymes*** . The authors have found that pcPNA-dsDNA complexes exhibit a different degree of DNA protection against cleaving/nicking activity of various isoschizomeric endonucleases under investigation (PleI, MlyI and N.BstNBI) depending on their type and mutual arrangement of PNA-binding and enzyme recognition/cleavage sites. The authors have also found that the pcPNA targeting to closely located PleI or BbsI recognition sites on dsDNA generates in some cases the nicking activity of these DNA cutters. At the same time, MlyI endonuclease, a PleI isoschizomer, does not exhibit any DNA nicking/cleavage activity, being completely blocked by the nearby

pcPNA binding. The results have general implications for effective pcPNA interference with the performance of DNA-processing proteins, thus being important for prospective applications of pcPNAs.

L10 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
2003:414003 Document No. 139:2888 Engineering of strand-specific, sequence-specific, DNA-nicking enzymes from ***restriction*** ***endonucleases*** . Heiter, Daniel; Lunnen, Keith; Wilson, Geoffrey G. (New England Biolabs, Inc., USA). U.S. Pat. Appl. Publ. US 2003100094 A1 20030529, 62 pp. (English). CODEN: USXXCO. APPLICATION: US 2002-223074 20020816. PRIORITY: US 2001-PV314386 20010823.
AB Methods are provided for converting into a sequence-specific, strand-specific, and location-specific DNA-nicking endonuclease, from a ***restriction*** ***endonuclease*** that recognizes an asym. DNA sequence, the endonuclease having two catalytic sites and one or more single sequence specific DNA-binding domains. In one embodiment the method requires inactivating one of the catalytic sites of the ***restriction*** ***endonuclease*** . In another embodiment, the ***restriction*** ***endonuclease*** is a dimer having a first and second subunit each comprising a sequence-specific DNA binding domain, a catalytic site, and a dimerization domain. The nicking endonuclease is formed from combining one subunit having an inactivated catalytic site and a second subunit having an inactivated DNA-binding domain. Thus, the type IIt ***restriction*** ***endonuclease*** from Bacillus brevis C (BbvCI) by site-specific mutagenesis of either the R1 subunit catalytic site (e.g., Glu-Val-Lys .fwdarw. Gly-Val-Lys) or the R2 catalytic site (e.g., Glu-Cys-Lys .fwdarw. Gly-Cys-Lys). The nicking endonuclease may be converted into a ***restriction*** ***endonuclease*** by the addn. of manganese cations in the digestion buffer.

L10 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
2003:360408 Document No. 139:18052 Artificial Site-Specific DNA-Nicking System Based on Common ***Restriction*** ***Enzymes*** Assisted by PNA Openers. Kuhn, Heiko; Hu, Yongbo; Frank-Kamenetskii, Maxim D.; Demidov, Vadim V. (Center for Advanced Biotechnology and Department of Biomedical Engineering, Boston University, Boston, MA, 02215, USA). Biochemistry, 42(17), 4985-4992 (English) 2003. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.
AB We report on the peptide nucleic acid (PNA)-directed design of a DNA-nicking system that enables selective and quant. cleavage of one strand of duplex DNA at a designated site, thus mimicking natural ***nickases*** and significantly extending their potential. This system exploits the ability of pyrimidine PNAs to serve as openers for specific DNA sites by invading the DNA duplex and exposing one DNA strand for oligonucleotide hybridization. The resultant secondary duplex can act as a substrate for a ***restriction*** ***enzyme*** , which ultimately creates a nick in the parent DNA. We demonstrate that several ***restriction*** ***enzymes*** of different types could be successfully used in the PNA-assisted system we developed. Importantly, the enzyme cleavage efficiency is basically not impaired on such artificially generated substrates, compared with the efficiency on regular DNA duplexes. Our design originates a vast class of semisynthetic rare-cleaving DNA ***nickases*** , which are essentially absent at present. In addn., we show that the site-specific PNA-assisted nicking of duplex DNA can be engaged in a rolling-circle DNA amplification (RCA) reaction. This new RCA format demonstrates the practical potential of the novel biomol. tool we propose for DNA technol. and DNA diagnostics.

L10 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
2003:333442 Artificial Site-Specific DNA-Nicking System Based on Common ***Restriction*** ***Enzymes*** Assisted by PNA Openers. Kuhn, Heiko; Hu, Yongbo; Frank-Kamenetskii, Maxim D.; Demidov, Vadim V. (Cent. Adv. Biotechnol., Dep. Biomed. Eng., Boston Univ., Boston, MA, 02215, USA). Biochemistry, 42(17), 4985-4992 (English) 2003. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.
AB We report on the peptide nucleic acid (PNA)-directed design of a DNA-nicking system that enables selective and quant. cleavage of one strand of duplex DNA at a designated site, thus mimicking natural ***nickases*** and significantly extending their potential. This system exploits the ability of pyrimidine PNAs to serve as openers for specific DNA sites by invading the DNA duplex and exposing one DNA strand for

oligonucleotide hybridization. The resultant secondary duplex can act as a substrate for a ***restriction*** ***enzyme*** , which ultimately creates a nick in the parent DNA. We demonstrate that several ***restriction*** ***enzymes*** of different types could be successfully used in the PNA-assisted system we developed. Importantly, the enzyme cleavage efficiency is basically not impaired on such artificially generated substrates, compared with the efficiency on regular DNA duplexes. Our design originates a vast class of semisynthetic rare-cleaving DNA ***nickases*** , which are essentially absent at present. In addn., we show that the site-specific PNA-assisted nicking of duplex DNA can be engaged in a rolling-circle DNA amplification (RCA) reaction. This new RCA format demonstrates the practical potential of the novelbiomol. tool we propose for DNA technol. and DNA diagnostics.

L10 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
2003:77452 Document No. 138:132166 Use of site-specific nicking endonucleases to create single-stranded regions and applications in molecular cloning thereof. Jack, William E.; Schildkraut, Ira; Menin, Julie Forney (New England Biolabs, Inc., USA). U.S. Pat. Appl. Publ. US 2003022317 A1 20030130, 34 pp. (English). CODEN: USXXCO. APPLICATION: US 2000-738444 20001215.

AB The present invention relates to the use of site-specific nucleic acid nicking enzymes to create single-stranded regions in duplex nucleic acids. Such single-stranded regions can take the form of gaps interior to the duplex, or terminal single-stranded regions. Single-stranded termini can be crafted to allow linkage of various elements via base-pairing with elements contg. a complementary single-stranded region. This joining is useful, for example, in an ordered, oriented assembly of DNA modules to create cloning or expression vectors. This joining is also useful in attaching detection probes and purifying DNA mols. contg. the single-stranded region. Gaps are useful in similar applications, including attaching detection or purifn. probes. Specifically, ***restriction*** ***endonuclease*** N.BstNBI is used to create vector cassettes with cohesive termini and inserting fragments with compatible cohesive ends (12nt or 18nt long). Several vectors are assembled from 2-4 DNA fragments contg. such cohesive ends.

L10 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
2002:926354 Document No. 138:182980 Tailoring the activity of ***restriction*** ***endonuclease*** PleI by PNA-induced DNA looping. Protozanova, Ekaterina; Demidov, Vadim V.; Soldatenkov, Viatcheslav; Chasovskikh, Sergey; Frank-Kamenetskii, Maxim D. (Center for Advanced Biotechnology, Boston University, Boston, MA, 02215, USA). EMBO Reports, 3(10), 956-961 (English) 2002. CODEN: ERMEAX. ISSN: 1469-221X. Publisher: Oxford University Press.

AB DNA looping is one of the key factors allowing proteins bound to different DNA sites to signal one another via direct contacts. We demonstrate that DNA looping can be generated in an arbitrary chosen site by sequence-directed targeting of double-stranded DNA with pseudocomplementary peptide-nucleic acids (pcPNAs). We designed pcPNAs to mask the DNA from cleavage by type IIs ***restriction*** ***enzyme*** PleI while not preventing the enzyme from binding to its primary DNA recognition site. Direct interaction between two protein mols. (one bound to the original recognition site and the other to a sequence-degenerated site) results in a totally new activity of PleI which produces a nick near the degenerate site. The PNA-induced nicking efficiency varies with the distance between the two protein-binding sites in a phase with the DNA helical periodicity. Our findings imply a general approach for the fine-tuning of proteins bound to DNA sites well sepd. along the DNA chain.

L10 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
2002:403841 Document No. 137:1491 Engineering nicking endonucleases from type IIs ***restriction*** ***endonuclease*** by mutating dimerization/cleavage domain. Kong, Huimin; Besnier, Caroline; Xu, Yan (New England Biolabs, Inc., USA). U.S. US 6395523 B1 20020528, 20 pp. (English). CODEN: USXXAM. APPLICATION: US 2001-872861 20010601.

AB The present invention relates to methods to engineer nicking endonucleases from existing Type IIs ***restriction*** ***endonuclease*** MlyI and AlwI, and the prodn. of the engineered nicking endonucleases. Specifically, the method comprises identifying a suitable double-stranded

nuclease followed by site-directed mutation of the dimerization interface responsible for double-stranded cleavage such that the mutated nuclease cleaves only one DNA strand at a specific location within or adjacent the recognition sequence. The mutation occurs by substituting one or more amino acid residues required for dimerization/cleavage, or swapping or substituting the region contg. the dimerization interface with a natural occurring nicking endonuclease, N.BstNBI.

L10 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
2002:87235 Document No. 136:130764 A strand-specific DNA ***nickase*** containing ***restriction*** ***endonuclease*** BPu10I subunits that cleaves asymmetric nucleotide sequences. Janulaitis, Arvydas; Stankevicius, Kornelijus; Lubys, Arvydas; Markauskas, Algimantas (Fermentas AB, Lithuania). Eur. Pat. Appl. EP 1176204 A1 20020130, 37 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2001-304891 20010605. PRIORITY: GB 2000-18120 20000724.
AB A strand-specific polynucleotide ***nickase*** comprising two subunits of the heteromeric ***restriction*** ***endonuclease*** , Bpu10I, and which recognizes an asym. nucleotide recognition sequence. One Bpu10I catalytic subunit is capable of cleaving one strand of a DNA duplex, and the second subunit is an inactivated endonuclease, incapable of cleaving the other strand of the DNA duplex. Furthermore, the catalytic domain is incapable of cleaving one strand of the DNA duplex in the absence of the other subunit and hence the second subunit might be important for target sequence recognition or for stabilizing the catalytic domain. The second Bpu10I subunit is inactivated by site-specific or non-specific mutagenesis. The ***nickase*** asym. recognition sequence contains 4 or more nucleotides. Test kits are another embodiment of the invention that include two ***nickases*** (each contg. a catalytic and inactivated subunit) and the catalytic subunit of each ***nickase*** recognizes a different strand of the asym. nucleotide sequence. These test kits contg. ***nickases*** can be used in generating circular single stranded DNA from circular double-stranded DNA. It can also be used to produce nested deletions in DNA and can be used in the generation of covalently closed linear DNA mols. Kits for producing covalently closed linear DNA comprising a ***nickase*** and a vector contg. a recognition sequence for a ***restriction*** ***endonuclease*** , flanked on each side by a pair of of ***nickase*** recognition sequence are provided. The ***nickase*** recognition sequences are inverted with respect to each other such that the ***nickase*** cleaves each strand of the vector on each side of the ***restriction*** ***endonuclease*** recognition site. One strand of the sequence between each pair of ***nickase*** recognition sequences contains a self-complementary sequence capable of forming a hairpin loop. Kits for prepg. vectors for use in ligation-independent cloning are another embodiment of the invention. A vector comprising a recognition sequence for a ***restriction*** ***endonuclease*** , flanked on each side by the ***nickase*** recognition sequence is provided in these kits. Specifically, the ***nickase*** recognition sequences are inverted with respect to each other such the ***nickase*** is capable of cleaving different strands of the vector on each side of the ***restriction*** ***endonuclease*** recognition sequence.

L10 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
2001:858308 Document No. 136:321259 Engineering a nicking endonuclease N.AlwI by domain swapping. Xu, Yan; Lunnen, Keith D.; Kong, Huimin (New England Biolabs, Beverly, MA, 01915, USA). Proceedings of the National Academy of Sciences of the United States of America, 98(23), 12990-12995 (English) 2001. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.
AB Changing enzymic function through genetic engineering still presents a challenge to mol. biologists. Here we present an example in which changing the oligomerization state of an enzyme changes its function. Type IIs ***restriction*** ***endonucleases*** such as AlwI usually fold into two sep. domains: a DNA-binding domain and a catalytic/dimerization domain. We have swapped the putative dimerization domain of AlwI with a nonfunctional dimerization domain from a nicking enzyme, N.BstNBI. The resulting chimeric enzyme, N.AlwI, no longer forms a dimer. Interestingly, the monomeric N.AlwI still recognizes the same sequence as AlwI but only cleaves the DNA strand contg. the sequence

5'-GGATC-3' (top strand). In contrast, the wild-type AlwI exists as a dimer in soln. and cleaves two DNA strands; the top strand is cleaved by an enzyme binding to that sequence, and its complementary bottom strand is cleaved by the second enzyme dimerized with the first enzyme. N.AlwI is unable to form a dimer and therefore nicks DNA as a monomer. In addn., the engineered nicking enzyme is at least as active as the wild-type AlwI and is thus a useful enzyme. To our knowledge, this is the first report of creating a nicking enzyme by domain swapping.

L10 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
1998:61424 Document No. 128:213917 Chlorella virus NY-2A encodes at least 12 DNA endonuclease/methyltransferase genes. Zhang, Yanping; Nelson, Michael; Nietfeldt, Joe; Xia, Yuannan; Burbank, Dwight; Ropp, Susan; Van Etten, James L. (Department of Plant Pathology, University of Nebraska, Lincoln, NE, 68583-0722, USA). Virology, 240(2), 366-375 (English) 1998. CODEN: VIRLAX. ISSN: 0042-6822. Publisher: Academic Press.
AB The 380-kb chlorella virus NY-2A genome is highly methylated; 45% of the cytosines are 5-methylcytosine (5mC) and 37% of the adenines are N6-methyladenine (6mA). Based on the sensitivity/resistance of NY-2A DNA to 80 methylation-sensitive DNA ***restriction*** ***endonucleases*** , the virus is predicted to encode at least 10 DNA methyltransferases: 7 6mA-specific methyltransferases, M.CviQI (GTmAC), M.CvQII (RmAR), M.CviQIII (TCGmA), M.CviQIV (GmATC), M.CviQV (TGCmA), M.CviQVI (GmANTC), and M.CviQVII (CmATG); and 3 5mC-specific methyltransferases, M.CviQVIII [RGmC(T/C/G)], M.CviQIX (mCC), and M.CviQX (mCGR). Five of the 6mA methyltransferase genes, M.CviQI, M.CviQIII, M.CviQV, M.CviQVI, and M.CviQVII, were cloned and sequenced. In addn., 2 site-specific endonuclease activities, R.CviQI (G/TAC) and NY2A-***nickase*** (R/AG), were detected in cell-free exts. from NY-2A virus-infected chlorella. Therefore, the NY-2A genome contains at least 12 DNA methyltransferase and endonuclease genes which, altogether, compose about 3-4% of the virus genome.

=> S L5 AND L8
L11 15 L5 AND L8

=> S L11 NOT L10
L12 5 L11 NOT L10

=> D 1-5 CBIB ABS

L12 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
2003:792787 Cloning and Sequencing of the Gene of Site-Specific ***Nickase*** N.BspD6I. Perevyazova, T. A.; Rogulin, E. A.; Zheleznaya, L. A.; Matvienko, N. I. (Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, 142290, Russia). Biochemistry (Moscow, Russian Federation)(Translation of Biokhimiya (Moscow, Russian Federation)), 68(9), 984-987 (English) 2003. CODEN: BIORAK. ISSN: 0006-2979. Publisher: MAIK Nauka/Interperiodica Publishing.
AB A fragment of chromosomal DNA from Bacillus species D6 contg. the gene of ***nickase*** N.BspD6I and the regions adjacent to its 5 - and 3 -ends was cloned and sequenced. The nucleotide sequence of the ***nickase*** gene, except of one neutral change, is homologous to the nicking ***endonuclease*** N.BstNBI gene sequenced by Higgens et al. (2001). After integration of a PCR-copy of the ***nickase*** gene into an expression vector pET28b under the control of the phage T7 promoter, specific nicking activity was detected in the lysates of transformed E. coli cells.

L12 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
2001:799183 Document No. 136:81818 Site-specific ***nickase*** from Bacillus species strain D6. Zheleznaya, L. A.; Perevyazova, T. A.; Alzhanova, D. V.; Matvienko, N. I. (Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, 142290, Russia). Biochemistry (Moscow, Russian Federation)(Translation of Biokhimiya (Moscow, Russian Federation)), 66(9), 989-993 (English) 2001. CODEN: BIORAK. ISSN: 0006-2979. Publisher: MAIK Nauka/Interperiodica Publishing.
AB Three site-specific ***endonucleases*** were found in thermophilic

strain Bacillus species D6. One of them, BspD6II, is an isoschizomer of Eco57I. The second, BspD6III, is present in the strain in very small amt.; therefore, it has not been characterized. This paper is devoted to the third, BspD6I, which recognizes pentanucleotide site 5'-GAGTC-3' and cleaves only one DNA strand at a distance of 4 nucleotides from the site in the 3'-direction in the chain with the GAGTC sequence, i.e., it behaves as a site-specific ***nickase*** . ***Nickase*** N.BspD6I cleaves one DNA strand only in double-stranded DNA and does not cleave single-stranded DNA. Site-specific methylase SscL1I (an isohypectomer of M.cntdot.HinfI) that methylates adenine in the sequence 5'-GANTC-3' prevents DNA hydrolysis by ***nickase*** BspD6I.

L12 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
1972:537726 Document No. 77:137726 Subcellular localization and characterization of DNA polymerases from rat liver and hepatomas. Baril, Earl; Laszlo, John (Duke Med. Cent., Durham, NC, USA). Advances in Enzyme Regulation, 9, 183-204 (English) 1971. CODEN: AEZRA2. ISSN: 0065-2571.
AB Previous studies from this laboratory described a correlation between DNA synthesis and DNA polymerase (I) activity in a variety of normal rat tissues, regenerating rat liver and a series of 12 types of rat hepatomas. I in these tissues, isolated by the Mantsavinos procedure, was sepd. into 2 peaks of activity by Sephadex G-200 gel filtration. A low mol. wt. fraction prefers native DNA primer, is the predominant component of normal rat liver, and increases markedly in regenerating liver. A fraction of high mol. wt. material which prefers denatured DNA as primer is present to a small extent in normal and regenerating rat liver but increases markedly in fetal rat liver (-6 days from birth), other developing tissues of the rat, rat hepatomas, rat renal tumors, and various human cancers. Subcellular fractionation of rat liver and selected Morris hepatomas showed that I activity preferring denatured DNA primer is assocd. with a cytoplasmic smooth membrane fraction in normal liver and is present in the nucleus, as well as with the smooth membranes from hepatomas tissues. The activity with these fractions from the progressed hepatoma 7777 was markedly increased. I preferring native DNA as primer is assocd. with the nucleus and ribosomes of rat liver and hepatoma. I assocd. with these subcellular fractions from rat liver and hepatoma tissues was partially purified and the properties of the polymerase compared. The ribosomal- and nuclear-assocd. polymerase of rat liver appears to be the same enzyme in that the phys. and enzymic properties of the purified enzyme are identical. The membrane-assocd. I, also present in nuclei of hepatomas, has different phys. and enzymic properties than the polymerase assocd. with both ribosomes and nuclei of liver. After purification both enzymes prefer activated DNA as primer. Purified membrane polymerase is free of nuclease activity but purified ribosome polymerase contains a small amt. of ***endonuclease*** activity that products single-strand breaks in DNA (" ***nickase*** "). All 4 deoxyribonucleoside triphosphates are required for normal activity of both polymerases but the purified polymerase from ribosomes has .apprx.25% maximal activity in the presence of a single deoxyribonucleoside triphosphate. This activity does not appear to catalyze terminal addn. to the primer since expts. with homodeoxyribopolymers demonstrated a requirement for base pairing with the template. It is suggested that the physiol. function of the polymerase assocd. with ribosomes and nuclei is in limited polymerization reactions such as DNA repair. The polymerase assocd. with smooth membranes, and present in the nuclei of proliferating tissues, may function in the replication reaction.

L12 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
1971:494756 Document No. 75:94756 Fluorometric assay for specific (***nickases***) and nonspecific endodeoxyribonucleases. Paoletti, C.; Le Pecq, Jean B. (Lab. Bio-Chim. Enzymol., Inst. Gustave Roussy, Villejuif, Fr.). Methods Enzymol., Volume 21, Issue Pt. D, 255-69. Editor(s): Colowick, Sidney P. Academic: New York, N. Y. (English) 1971. CODEN: 18HWA8.
AB The assay is based on the ability of ethidium bromide, a double-stranded DNA intercalating dye, to unwind DNA strands. Max. unwinding can be reached for a satg. amt. of dye. Thus, on a closed circle of DNA a first break results in an increased amt. of dye able to be bound at satn., and in a population of closed circles the no. broken can be calcd. The no. of nicks induced by an ***endonuclease*** or any breaking agent can thereby be calcd. The use of this assay, the mechanism of action of an

endonuclease , and the advantages and disadvantages of the assay are discussed.

L12 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
1971:430319 Document No. 75:30319 Cofractionation of " ***nickase*** " with rat liver DNA polymerase. Baril, Earl F.; Brown, Oliver; Laszlo, John (Med. Cent., Duke Univ., Durham, NC, USA). Biochemical and Biophysical Research Communications, 43(4), 754-9 (English) 1971. CODEN: BBRCA9. ISSN: 0006-291X.
AB DNA polymerase partially purified from rat liver nuclei or ribosomes contains alk. ***endonuclease*** that produces single strand breaks (nicks) in rat liver nuclear DNA. The nuclease activity is specific for DNA and is active with both native and denatured DNA as substrates. The ***endonuclease*** has maximal activity in the presence of 15 mM Mg2+ at pH 9.0, but is inactive in the presence of Ca2+ at concns. that also inactivate the DNA polymerase. The 3' -OH primer ends produced by the ***endonuclease*** may account, in part, for the native DNA primer preference of the polymerase obsd. in vitro.

=> S L4 AND L8
L13 8 L4 AND L8

=> S L13 NOT (L10,L11)
L14 3 L13 NOT ((L10 OR L11))

=> D 1-3 CBIB ABS

L14 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
2003:511517 Document No. 139:80146 Tsunami chain reaction method for geometric amplification of DNA. Denton, Richard V.; Bowlby, James O., Jr. (USA). PCT Int. Appl. WO 2003054214 A2 20030703, 42 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US39063 20021206. PRIORITY: US 2001-PV339796 20011210.
AB The present invention relates to methods, kits and compns. for amplifying and detecting a target nucleic acid by releasing multiple free copies of probes contg. a sequence in common with the target nucleic acid. This is accomplished by using at least two sets of sepd. probes, one which includes a portion contg. a sequence complementary to the target nucleic acid, and the other which includes a portion in common with the target nucleic acid. Repeated hybridization and release of probes in the probe sets results in a rapid geometric increase in the free copies of the target nucleic acid, resulting in a capability to detect the target nucleic acid with very high sensitivity. The mechanism works by using properties of certain enzyme to "nick", i.e., hydrolyze the phosphodiester bond, of one strand of a nucleotide duplex without cleaving the other strand. This property can be used to release tags from probes which in turn hybridize to other nickable probes to release addnl. tags functionally equiv. to the original target DNA< thereby causing a geometric growth in the no. of copies of the target DNA. Detection occurs using a variety of labels or other means. The technol., named Tsunami Chain Reaction (TCR), can be deployed in an array format to offer massively parallel assays for many genes with fluorescent detection just like regular microarrays but without for example dual dye-based comparisons. On the other hand, TCR can be made to be a homogeneous assay and with greater precision in quantification just like the 5'- ***nuclease*** assay. TCR on arrays is thus efficient for carrying out gene expression profiling. The invention allows detection of extremely small concns. of target DNA, down to a single mol. in a test vol.

L14 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
1989:455046 Document No. 111:55046 Identification of nuclear proteins that specifically interact with adeno-associated virus type 2 inverted terminal repeat hairpin DNA. Ashktorab, Hassan; Srivastava, Arun (Sch. Med., Indiana Univ., Indianapolis, IN, 46202, USA). Journal of Virology, 63(7),

3034-9 (English) 1989. CODEN: JOVIAM. ISSN: 0022-538X.
AB A palindromic hairpin duplex contg. the inverted terminal repeat sequence of adeno-assocd. virus type 2 (AAV) DNA was used as a substrate in gel retardation assays to detect putative proteins that specifically interact with the AAV hairpin DNA structures. Nuclear proteins were detected in exts. prepd. from human KB cells coinfected with AAV and adenovirus type 2 that interacted with the hairpin duplex but not in nuclear exts. prepd. from uninfected, AAV-infected, or adenovirus type 2-infected KB cells. The binding was specific for the hairpin duplex, since no binding occurred with a double-stranded DNA duplex with the identical nucleotide sequence. Furthermore, in competition expts., the binding could be reduced with increasing concns. of the hairpin duplex but not with the double-stranded duplex DNA with the identical nucleotide sequence. S1 ***nuclease*** assays revealed that the binding was sensitive to digestion with the enzyme, whereas the protein-bound hairpin duplex was resistant to digestion with S1 ***nuclease*** . The nucleotide sequence involved in the protein binding was localized within the inverted terminal repeat of the AAV genome by methylation interference assays. These nuclear proteins may be likely candidates for the pivotal enzyme ***nickase*** required for replication or resoln. (or both) of single-stranded palindromic hairpin termini of the AAV genome.

L14 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
1974:56954 Document No. 80:56954 Separation and characterization of protein factor of DNA polymerase B from developing rat brain. Chiu, Jen-Fu; Sung, S. C. (Dep. Psychiatry, Univ. British Columbia, Vancouver, BC, Can.). Biochimica et Biophysica Acta, 331(1), 54-60 (English) 1973. CODEN: BBACAQ. ISSN: 0006-3002.
AB DNA polymerase B extracted from developing rat brain and subjected to (NH4)2SO4 fractionation and column chromatography on DEAE-cellulose lost its activity after Sephadex G-200 gel filtration due to loss of an activating factor. The partially inactivated DNA polymerase was markedly stimulated by the addition of this factor. The ability of the activating factor to enhance DNA polymerase B was destroyed by its treatment with a proteolytic enzyme as well as by heating. The effect of the activating factor on polymerase B does not appear to be a consequence of either ***nuclease*** or " ***nickase*** " activity.

```
=> S L9 AND L6
L15       1089 L9 AND L6

=> S L9(4A)L6
L16         78 L9(4A)L6

=> S L16 AND (MUTATE OR MUTATION OR INACTIVATE)
           681 MUTATE
           107 MUTATES
           784 MUTATE
                 (MUTATE OR MUTATES)
        194666 MUTATION
        125215 MUTATIONS
        241458 MUTATION
                 (MUTATION OR MUTATIONS)
         10466 INACTIVATE
          5464 INACTIVATES
         15557 INACTIVATE
                 (INACTIVATE OR INACTIVATES)
L17         21 L16 AND (MUTATE OR MUTATION OR INACTIVATE)

=> S L17 NOT (L10,L11,L14)
L18         20 L17 NOT ((L10 OR L11 OR L14))

=> D 1-20 TI

=> E JANULAITIS A/AU
=> S E3-E5
           92 "JANULAITIS A"/AU
            6 "JANULAITIS A A"/AU
           19 "JANULAITIS ARVYDAS"/AU
L19       117 ("JANULAITIS A"/AU OR "JANULAITIS A A"/AU OR "JANULAITIS ARVYDAS
```

```
              "/AU)

=> E STANKEVICIUS/AU
=> S E11,E12
             3 "STANKEVICIUS K"/AU
             5 "STANKEVICIUS KORNELIJUS"/AU
L20          8 ("STANKEVICIUS K"/AU OR "STANKEVICIUS KORNELIJUS"/AU)

=> E LUBYS A/AU
=> S E3,E4
             8 "LUBYS A"/AU
            13 "LUBYS ARVYDAS"/AU
L21         21 ("LUBYS A"/AU OR "LUBYS ARVYDAS"/AU)

=> E MARKAUSKAS/AU
=> S E4,E5
             2 "MARKAUSKAS A"/AU
             2 "MARKAUSKAS ALGIMANTAS"/AU
L22          4 ("MARKAUSKAS A"/AU OR "MARKAUSKAS ALGIMANTAS"/AU)

=> S L19,L20,L21,L22
L23        126 (L19 OR L20 OR L21 OR L22)

=> S L23 AND L6
L24         85 L23 AND L6

=> S L24 AND (L7,L8)
L25          1 L24 AND ((L7 OR L8))

=> D CBIB ABS

L25  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2003 ACS on STN
2002:87235   Document No. 136:130764  A strand-specific DNA   ***nickase***
     containing   ***restriction***     ***endonuclease***   BPul0I subunits
     that cleaves asymmetric nucleotide sequences.    ***Janulaitis, Arvydas***
     ;   ***Stankevicius, Kornelijus***  ;   ***Lubys, Arvydas***  ;
       ***Markauskas, Algimantas***   (Fermentas AB, Lithuania). Eur. Pat. Appl.
     EP 1176204 A1 20020130, 37 pp.  DESIGNATED STATES: R:  AT, BE, CH, DE, DK,
     ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO.
     (English).  CODEN: EPXXDW.  APPLICATION: EP 2001-304891 20010605.
     PRIORITY: GB 2000-18120 20000724.
AB   A strand-specific polynucleotide   ***nickase***   comprising two subunits
     of the heteromeric   ***restriction***     ***endonuclease***  , Bpul0I,
     and which recognizes an asym. nucleotide recognition sequence.  One Bpul0I
     catalytic subunit is capable of cleaving one strand of a DNA duplex, and
     the second subunit is an inactivated endonuclease, incapable of cleaving
     the other strand of the DNA duplex.  Furthermore, the catalytic domain is
     incapable of cleaving one strand of the DNA duplex in the absence of the
     other subunit and hence the second subunit might be important for target
     sequence recognition or for stabilizing the catalytic domain.  The second
     Bpul0I subunit is inactivated by site-specific or non-specific
     mutagenesis.  The   ***nickase***   asym. recognition sequence contains 4
     or more nucleotides.  Test kits are another embodiment of the invention
     that include two   ***nickases***   (each contg. a catalytic and
     inactivated subunit) and the catalytic subunit of each   ***nickase***
     recognizes a different strand of the asym. nucleotide sequence.  These
     test kits contg.   ***nickases***   can be used in generating circular
     single stranded DNA from circular double-stranded DNA.  It can also be
     used to produce nested deletions in DNA and can be used in the generation
     of covalently closed linear DNA mols.  Kits for producing covalently
     closed linear DNA comprising a   ***nickase***   and a vector contg. a
     recognition sequence for a   ***restriction***     ***endonuclease***  ,
     flanked on each side by a pair of of   ***nickase***   recognition
     sequence are provided.  The   ***nickase***   recognition sequences are
     inverted with respect to each other such that the   ***nickase***
     cleaves each strand of the vector on each side of the   ***restriction***
       ***endonuclease***   recognition site.  One strand of the sequence between
     each pair of   ***nickase***   recognition sequences contains a
     self-complementary sequence capable of forming a hairpin loop.  Kits for
     prepg. vectors for use in ligation-independent cloning are another
     embodiment of the invention.  A vector comprising a recognition sequence
```

for a ***restriction*** ***endonuclease*** , flanked on each side by the ***nickase*** recognition sequence is provided in these kits. Specifically, the ***nickase*** recognition sequences are inverted with respect to each other such the ***nickase*** is capable of cleaving different strands of the vector on each side of the ***restriction*** ***endonuclease*** recognition sequence.

=> S L22 AND L9
L26 1 L22 AND L9

=> S L26 NOT L25
L27 0 L26 NOT L25

	L #	Hits	Search Text	DBs
1	L1	40849	RESTRICTION ADJ (ENDONUCLEASE OR ENZYME)	USPAT ; US-PG PUB
2	L2	34	NICKASE	USPAT ; US-PG PUB
3	L3	29	L1 AND L2	USPAT ; US-PG PUB
4	L4	8	L1 NEAR10 L2	USPAT ; US-PG PUB
5	L5	27846	ENDONUCLEASE	USPAT ; US-PG PUB
6	L6	28	L5 AND L2	USPAT ; US-PG PUB
7	L7	3	L6 NOT L3	USPAT ; US-PG PUB